PEMPHIGUS VULGARIS

I. Analysis of β_{2A} and β_{2M} Serum Proteins by Immunoelectrophoresis*

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With the development of immunoelectrophoresis, serum proteins can now be more extensively analyzed. This report will deal with the application of the method of immunoelectrophoresis to the analysis of serum from patients with pemphigus. According to Lever (1) who reported the electrophoretic analysis of serum proteins in pemphigus, there is a decrease in serum albumin and an increase in α_1, α_2, β_2 and γ globulins Pozzo and Hofman (2) analyzed electrophoretically the serum proteins in pemphigus vulgaris and reported essentially the same findings as did Lever.

Grabar and Williams (3) demonstrated by immunoelectrophoresis that there are many more protein fractions in serum than can be analyzed by standard electrophoresis. Among the many new fractions, they identified two globulins which could not be demonstrated by previous analytical methods. These globulins were different from classical γ globulin in chemical and physical properties and were designated β_{2A} and β_{2M} globulins. This report will be concerned only with these two globulins, β_{2A} and β_{2M}. The other fractions demonstrated by immunoelectrophoresis are presently under investigation and will not be discussed.

METHODS, PROCEDURES AND MATERIALS

Venous blood was taken from fifteen patients with bullous dermatoses diagnosed as pemphigus vulgaris and from fifteen normal individuals. Three of the patients had what might be diagnosed as pemphigoid in that they had a chronic, generalized, tense non-grouped, bullous eruption without involvement of the mucous membrane, and their general health was unimpaired except for chronic physical changes which might have been associated with their advanced age (60 years and over). These cases were not classified on the basis of the presence or absence of acantholysis because of the difficulty of demonstrating acantholysis unless a very large number of biopsies are performed on each patient. All pemphigus patients had active lesions but cases No. 2, 4, 7 and 8 were receiving oral steroids at the time that their blood serum samples were taken.

Serum from fifteen normal individuals was analyzed. The list of donors included five persons between 20 to 30 years of age and 10 individuals whose ages ranged from 30 to 70 years. One third of the normal group were females. All fifteen were in apparently good health.

The venous blood was allowed to clot at room temperature for two hours. Then the clotted blood was refrigerated at 4° C for 12 hours, after which the serum was separated twice by centrifugation at 1500 RPM for 15 minutes and then stored in a −20° C deep freeze until it was used.

Agar immunoelectrophoretic microscopic slides were prepared by placing 2 ml. of preheated 2% agar which contained phosphate-borate buffer (pH 8.6 and ionic strength 0.05) on a microscopic slide (2.5 x 7.5 cm.). The procedure was carried out on a leveled board. The agar was then allowed to solidify and two wells for the antigens (the normal person's serum or the patient's serum) and a trough for the antiserum was cut by pre-set cutter to give a consistent shaped trough and wells (figure 1).

Before applying current to the agar microscopic slides 0.005 ml. of antigen (serum) was placed in the well by means of a syringe. The slides were then placed for electrophoresis and the voltage and amperes which were measured across each slide were approximately 54 volts at 2.8 ma. The phosphate-borate buffer solution was used at the ends of the slides to allow a uniform electric contact with each slide. The time allowed for electrophoresis was 60 minutes in a 4° C cold room.

After electrophoresis the slides were brought to room temperature and the middle trough was removed with a probe. Antiserum (0.06 ml.) was placed in the central trough. Again this procedure was done on a leveled board. The diffusion of the antiserum was developed over a 16 to 24 hour period in a moist atmosphere in large dishes at room temperature. Then the agar slides were continuously washed with normal saline solution in a 4° C cold room for 5 days in order to wash away excess unreacted proteins. The agar was dried on the slide by placing No. 1 Whatman filter paper over the agar and exposing the slide to air at room temperature. The precipitation lines were stained with Amido Schwartz 10B dye solution (400 ml.

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ethyl alcohol, 400 ml. distilled water, 80 ml. glacial acetic acid and 1 gram of Amido Schwartz 10B) for approximately 10 minutes. The stained agar slides were then washed for three to six hours with the same solution without the Amido Schwartz 10B.

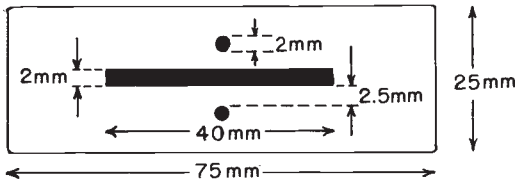

FIG. 1

TABLE I

2 fold antigen con. undiluted antiserum		undiluted antigen undiluted antiserum		1:2 diluated antigen undiluted antiserum		1:4 diluted antigen undiluted antiserum		Cases	
β_{2A}	β_{2M}	β_{2A}	β_{2M}	β_{2A}	β_{2M}	β_{2A}	β_{2M}		
+	±	+	−	+	−	±	−	1	pemphigus
+	+	+	+	+	±	+	−	2	
+	−	+	−	+	−	+	−	3	
±	+	−	±	−	−	−	−	4	
+	±	+	−	+	−	±	−	5	
+	±	+	−	+	−	+	−	6	
±	+	−	±	−	−	−	−	7	
±	+	−	±	−	−	−	−	8	
+	±	+	−	+	−	−	−	9	
+	−	+	−	+	−	±	−	10	
+	±	+	−	+	−	−	−	11	
+	−	+	−	+	−	±	−	12	
+	±	+	±	+	−	+	−	1	pemphigoid
+	±	+	−	+	−	+	−	2	
+	+	+	±	+	−	+	−	3	
±	+	−	+	−	±	−	−	1	normal
+	+	−	+	−	−	−	−	2	
±	+	−	+	−	±	−	−	3	
+	+	−	+	−	±	−	−	4	
±	+	−	+	−	−	−	−	5	
±	+	−	+	−	−	−	−	6	
+	+	−	+	−	+	−	−	7	
+	+	−	+	−	+	−	−	8	
±	+	−	+	−	±	−	−	9	
+	+	−	+	−	±	−	−	10	
±	+	−	+	−	−	−	−	11	
±	+	−	+	−	−	−	−	12	
+	+	−	+	−	−	−	−	13	
±	+	−	+	−	±	−	−	14	
+	+	+	+	±	−	−	−	15	

+: precipitating line is definitely present
±: precipitating line is faintly seen
−: no precipitating line seen

Each serum antigen sample was run in duplicate. In the first run the serum was in the left hand well and in the second run it was in the right hand well. This was done in order to avoid inconsistencies in the agar preparation which might affect the positions of the precipitating lines. The following dilutions of antigen were used: undiluted, 1:2 dilution (with normal saline) and 1:4 dilution. The antigen was also concentrated two fold by evaporation to ½ its normal volume. The antiserum was always used undiluted. The antiserum was obtained from the Pasteur Institute and was antihuman serum horse serum No. 13416.

RESULTS

Table I summarizes the results of these experiments. Particularly noteworthy is the increase of β_{2A} and the decrease of β_{2M} globulins in the abnormal serums. Under the conditions of microimmunoelectrophoresis as outlined in the procedure, it will be noted that in normals, the β_{2M} fraction was still present up to 1:2 fold antigen dilution, while the β_{2A} was detected only when the antigen was concentrated two fold. In patients with pemphigus and pemphigoid, on the other hand, the β_{2M} fraction was decreased by approximately ¾ compared to normals while the β_{2A} fraction was increased by approximately 8 fold. Figure 2 shows the immunoelectrophoretic patterns of the above findings in a normal and pemphigus patient.

DISCUSSION

Grabar and Williams (3) demonstrated by immunoelectrophoresis two protein fractions which had not been previously demonstrated by electrophoresis. One of these fractions had a high molecular weight (S_F 19) and had a carbohydrate content of approximately 10.66%; this was designated β_{2M}. The second fraction had a sedimentation constant (S_F) of 7 and on chemical analysis had a carbohydrate content of approximately 9.8%; this was labelled β_{2A} globulin. Changes in β_{2A} and β_{2M} fractions of serum proteins could not be demonstrated by electrophoresis because these fractions were masked by the β and γ globulins (4). It was recently demonstrated (5) by the ultracentrifuge technic that γ globulin had two fractions with different sedimentation constants, one being a S_F 7 protein and the other a S_F 19. It was also found that the S_F 19 fraction had approximately four times the carbohydrate content of the S_F 7 fraction. The S_F 19 γ globulin identified by the ultracentrifuge technic (5) appears to be the same as the

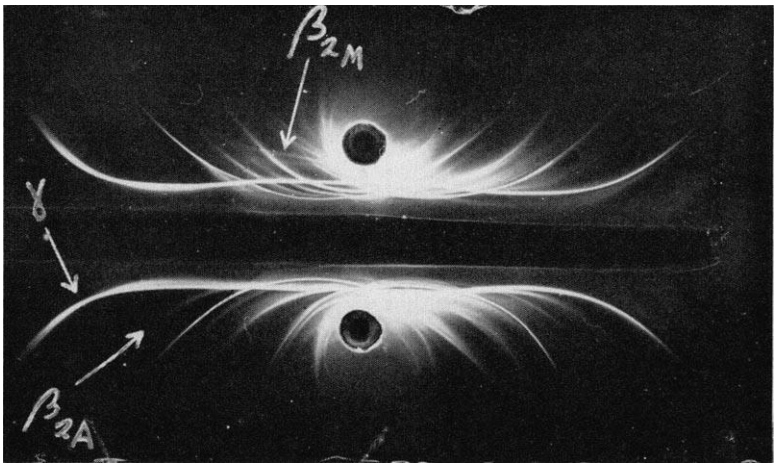

FIG. 2. Note the presence of the β_{2A} and the absence of the β_{2M} fraction in the lower immunoelectrophoretic pattern, which illustrates pemphigus vulgaris. The upper pattern is of normal serum and has the horizontal β_{2M} fraction indicated.

S_F 19 globulin (β_{2M}) identified by immunoelectrophoresis in that both have the same high carbohydrate content. Also the γ globulin demonstrated by immunoelectrophoresis has only one S_F constant, that is 7, and a carbohydrate content of 2.58% which is the same as the S_F 7γ globulin as demonstrated by the ultracentrifuge. The β_{2A} fraction had not been previously clearly recognized as it migrated on electrophoresis in the area between the β and γ globulins, also with analysis by the ultracentrifuge it could be confused with the S_F 7γ globulin. These fractions (β_{2A} [S_F 7] and γ globulin [S_F 7]) can be chemically differentiated, as β_{2A} globulin has a 10.66% carbohydrate content while γ globulin carbohydrate content is 2.58%. It now appears that these β globulins have a function similar to γ globulin.

In the past only the γ globulin was considered to be involved in the immunoglobulin system, but it now appears that β_{2A} and β_{2M} globulins are also concerned. In agammaglobulinemia (6) immunoelectrophoresis demonstrated that the γ, β_{2A} and β_{2M} fractions are absent or markedly decreased. Heremans (7) reports instances of acquired immune paralysis in which any one of the three components γ, β_{2A} or β_{2M} may be absent or decreased while the other fractions are normal or increased. Heremans concluded that various protein synthesizing mechanisms provide the organisms with a broad spectrum of antibodies and at least these three γ, β_{2A} and β_{2M} globulins may carry antibodies.

β_{2A} and β_{2M} abnormalities have been reported in various disorders. In cirrhosis of the liver (4, 8) and in certain types of multiple myeloma (4, 9) there is an increase in the β_{2A} fraction. In Waldenström's (10) macroglobulinemic purpura the β_{2M} fraction is markedly increased and has been implicated in the production of the petechiae.

Each of the three main immunoglobulins: γ, β_{2A} and β_{2M} may give rise to so-called paraproteins. These paraproteins are probably qualitatively very different from the normal fractions. It has been noted (7) that an increase in one paraprotein is often associated with a depression of the synthesis of other immunoglobulins. The immunochemistry of these paraproteins is so similar to that of normal immunoglobulins that the paraproteins precipitate when exposed to antihuman serum horse serum.

Results of our study would indicate that in clinically active pemphigus components β_{2A} and β_{2M} are altered. Cases No. 2, 4, 7 and 8 have been receiving steroids orally and do not show consistent immunoelectrophoretic patterns. Whether the increase in the β_{2A} fraction noted in pemphigus results from an increase in the quantity of normal β_{2A} globulin, or indicates the formation of a paraprotein remains to be determined. The decrease in the β_{2M} fraction may be due to immune paralysis of the system which produces the β_{2M} globulin, or to excessive destruction of β_{2M}.

It is interesting to note that the serum protein alterations as demonstrated by immunoelectrophoresis are the same in patients having an

atypical eruption which might be designated pemphigoid as in the serum of patients having classical pemphigus vulgaris.

SUMMARY

Sera from patients with pemphigus were examined by immunoelectrophoresis and compared with sera from normal individuals. Significant and consistent deviations from normal were demonstrated in that the β_{2M} fraction was decreased while the β_{2A} fraction was increased.

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